U.S. Serial No.: New Application Atty. Dkt. No.: 03659.00009
Inventor: Mark J. COOPER et al.
Title: LYOPHILIZABLE COMPACTED NUCLEIC ACIDS

7.5 µg DNA

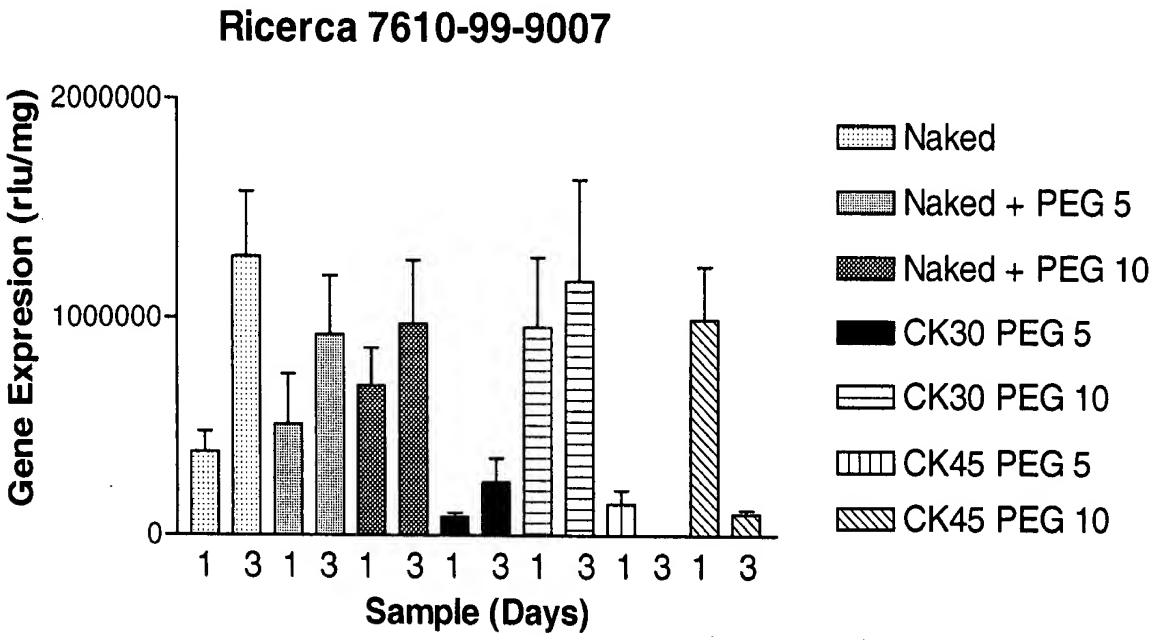

Fig. 1

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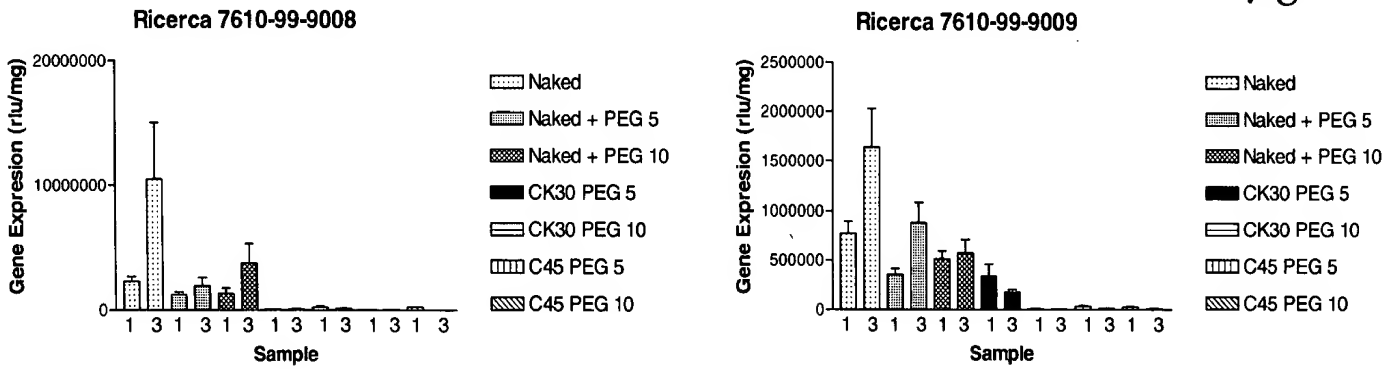


Fig. 2

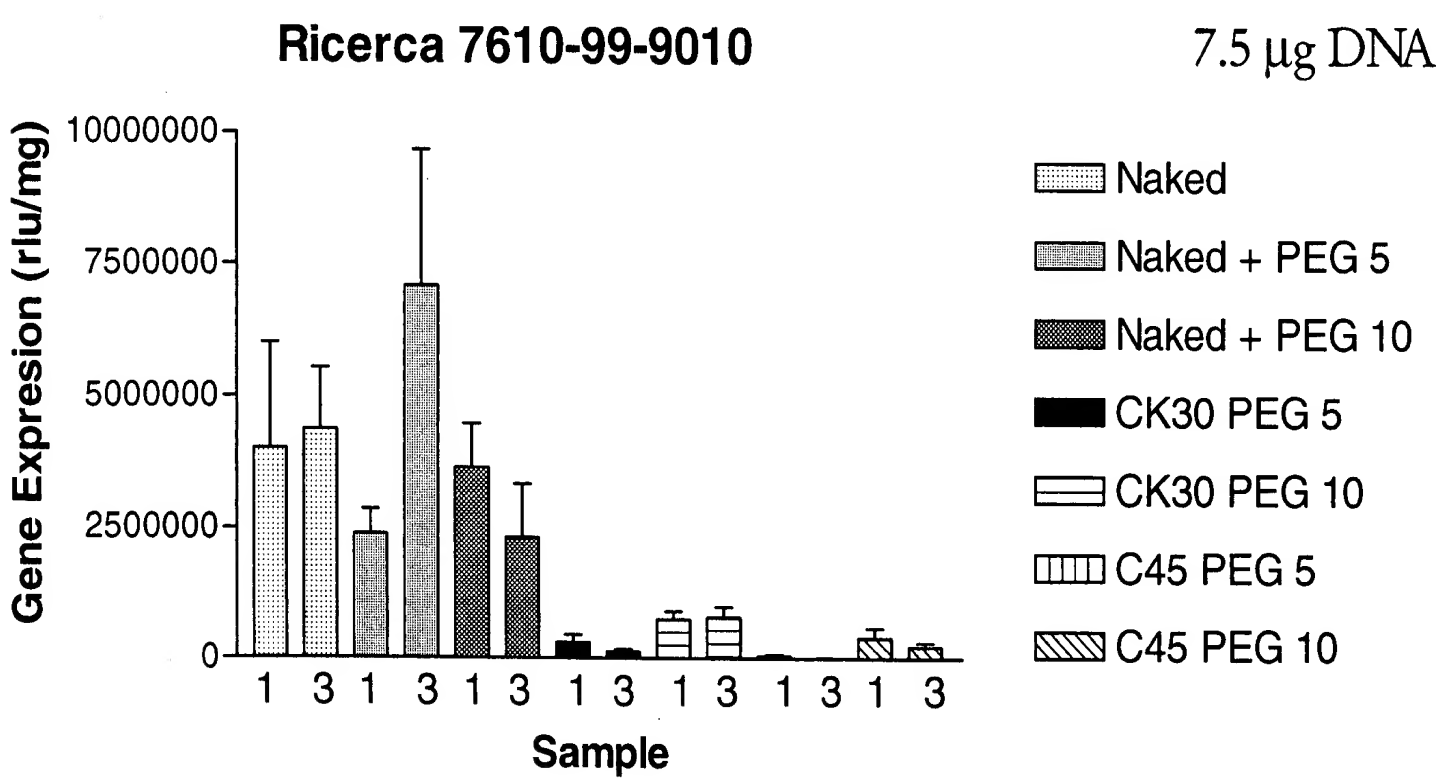


Fig. 3

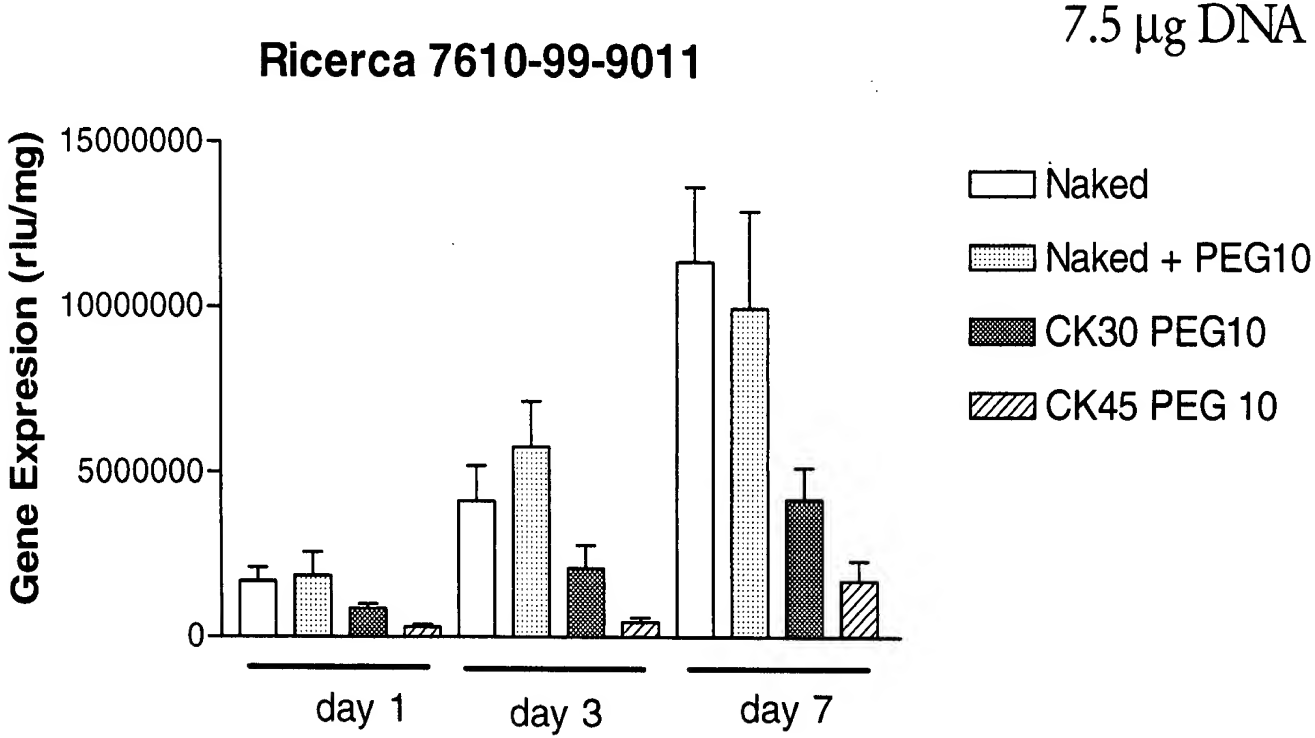


Fig. 4

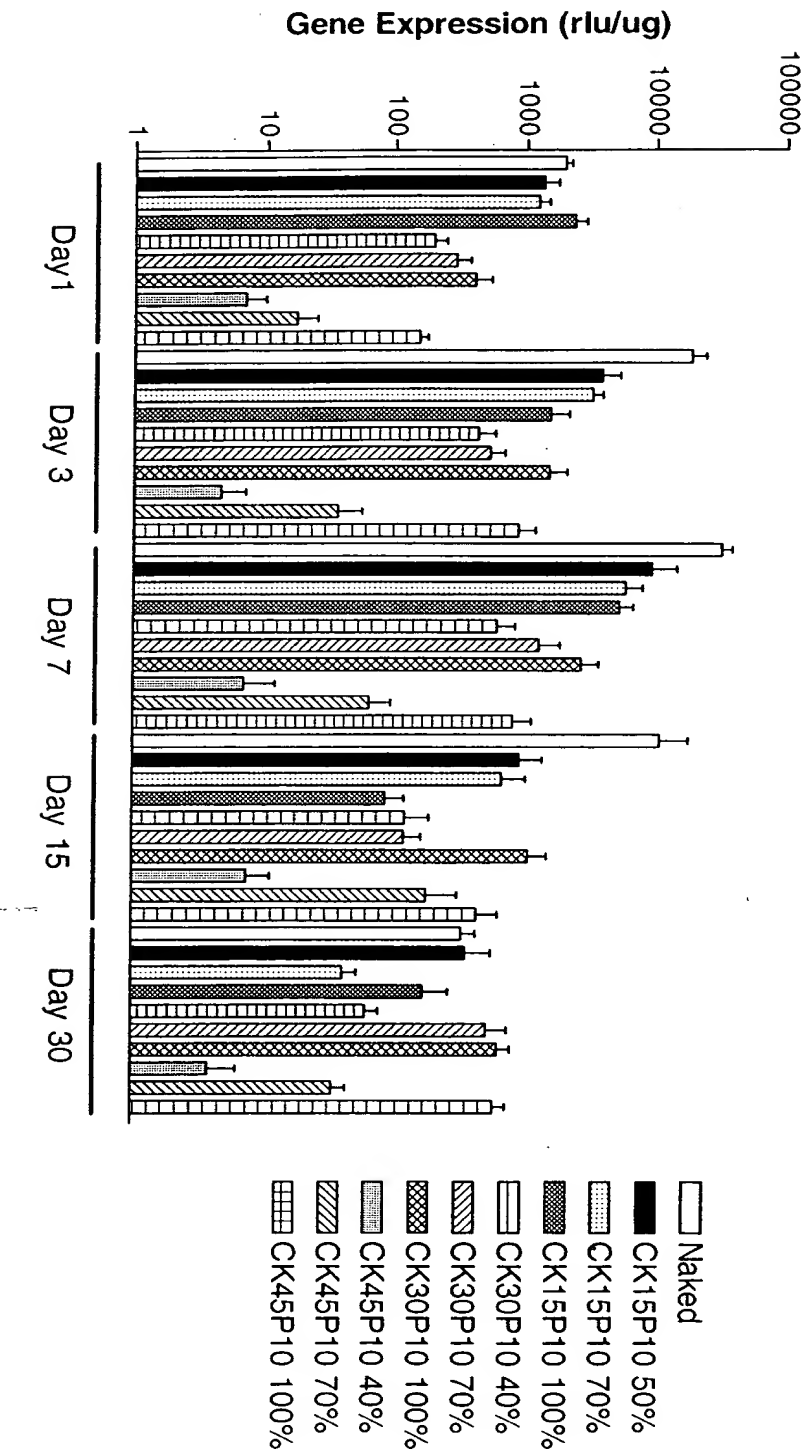

Gene Expression (rlu/ug)
100000
10000
1000
100
10
1
Day1
Day 3
Day 7
Day 15
Day 30
Ricerca 7610-99-9013
7.5 µg DNA
Naked
CK15P10 50%
CK15P10 70%
CK15P10 100%
CK30P10 40%
CK30P10 70%
CK30P10 100%
CK45P10 40%
CK45P10 70%
CK45P10 100%

Fig. 5

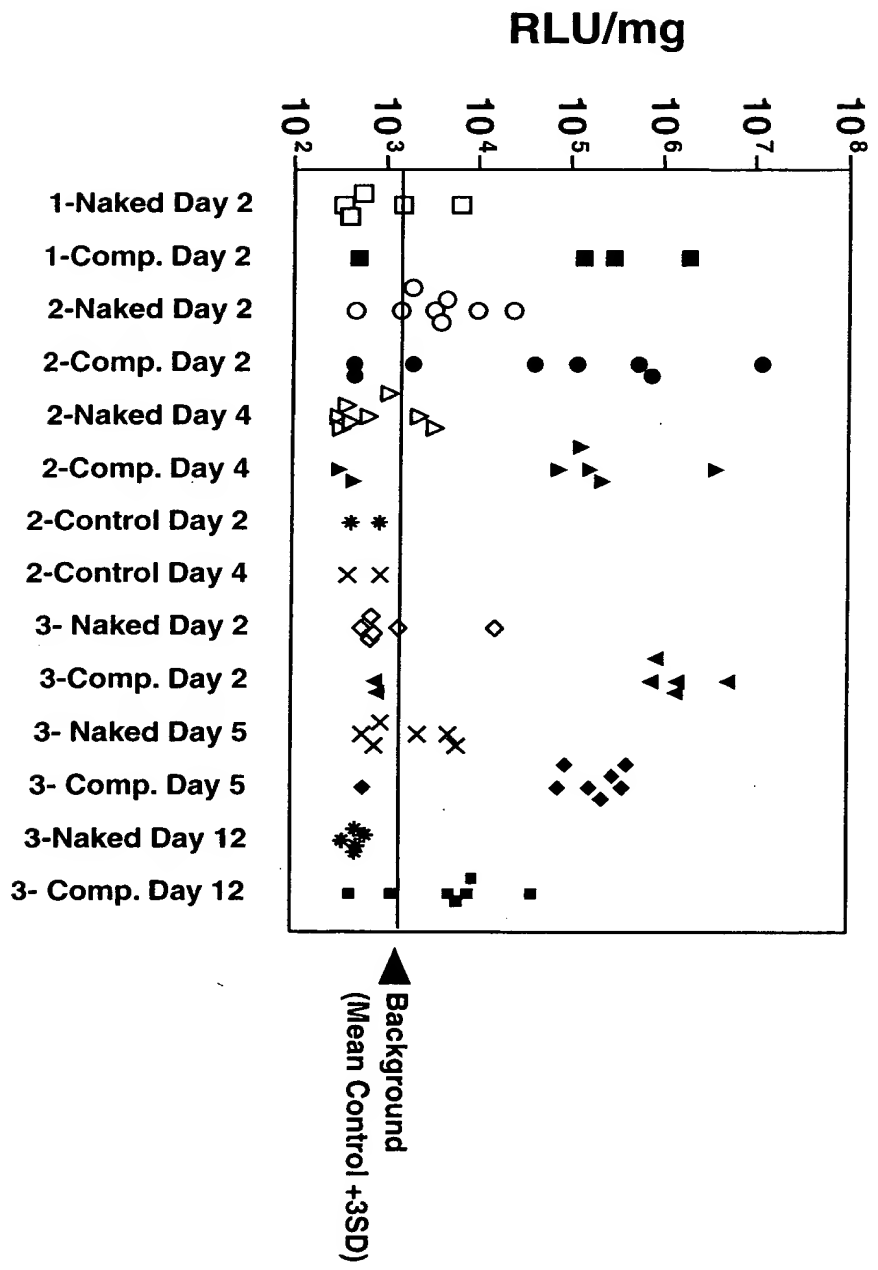

RLU/mg
10^2
10^3
10^4
10^5
10^6
10^7
10^8
1-Naked Day 2
1-Comp. Day 2
2-Naked Day 2
2-Comp. Day 2
2-Naked Day 4
2-Comp. Day 4
2-Control Day 2
2-Control Day 4
3- Naked Day 2
3-Comp. Day 2
3- Naked Day 5
3- Comp. Day 5
3-Naked Day 12
3- Comp. Day 12
Background
(Mean Control +3SD)

Fig. 6

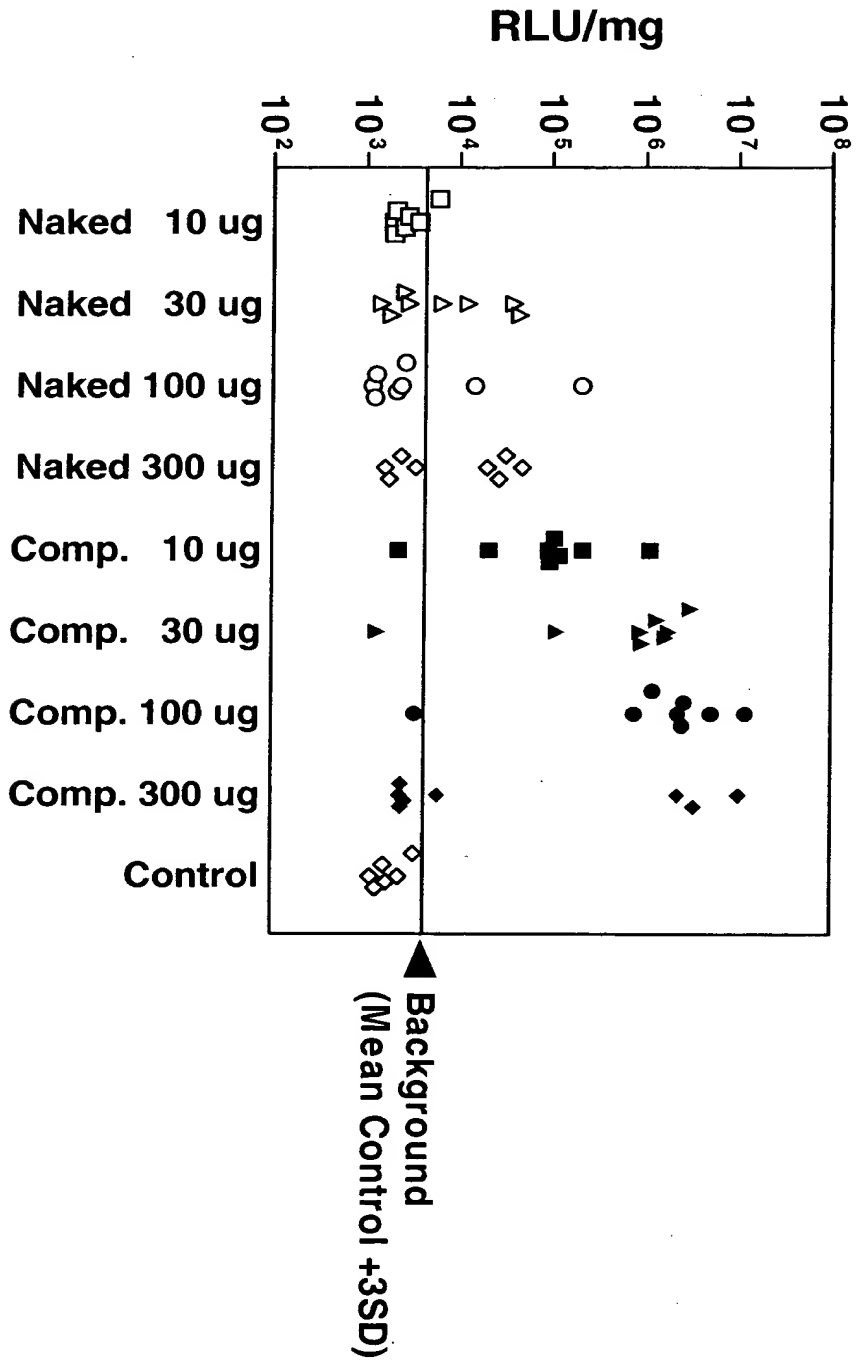

RLU/mg
10^2
10^3
10^4
10^5
10^6
10^7
10^8
Naked 10 ug
Naked 30 ug
Naked 100 ug
Naked 300 ug
Comp. 10 ug
Comp. 30 ug
Comp. 100 ug
Comp. 300 ug
Control
Background
(Mean Control +3SD)

Fig. 7A

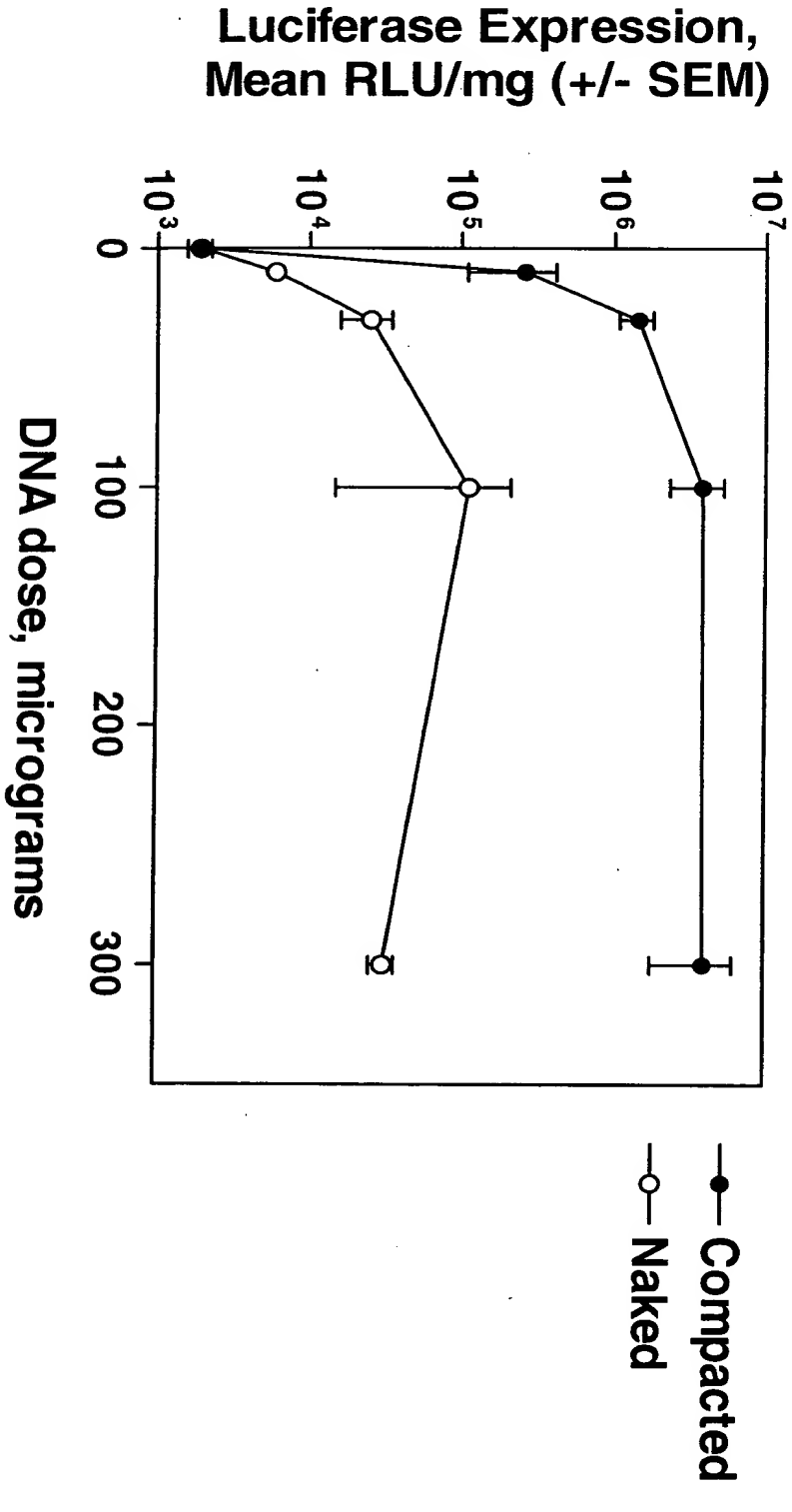

Luciferase Expression,
Mean RLU/mg (+/- SEM)
10^3
10^4
10^5
10^6
10^7
0
100
200
300
DNA dose, micrograms
Compacted
Naked

Fig. 7B

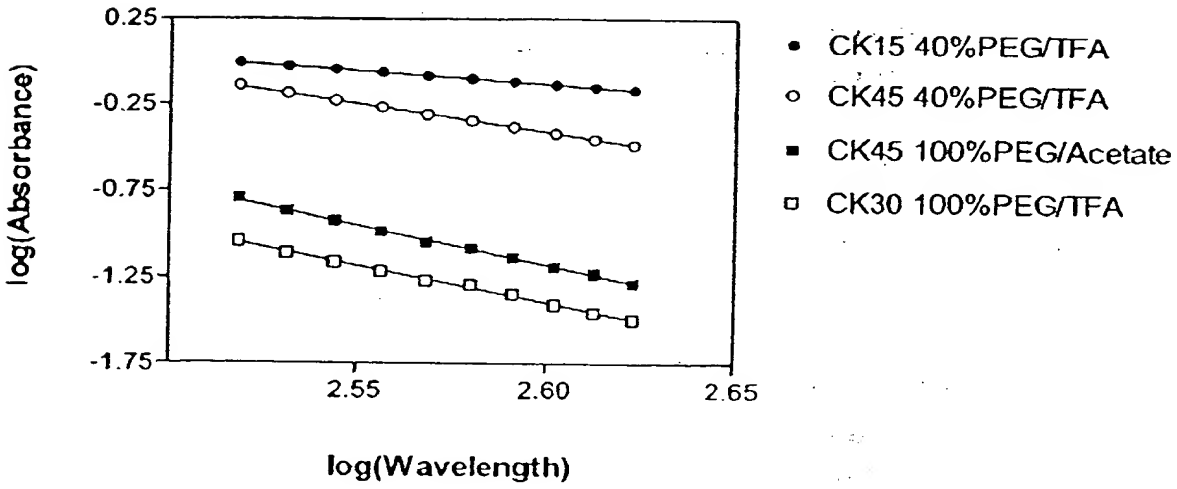
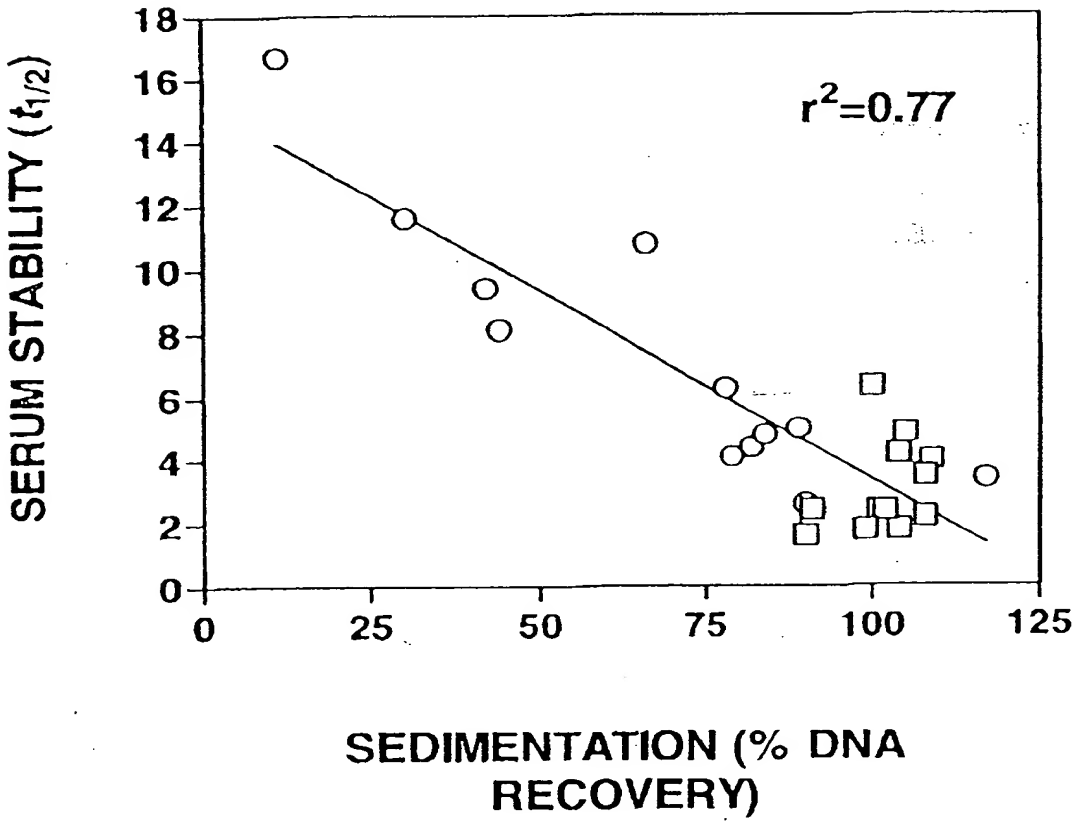

0.25
-0.25
-0.75
-1.25
-1.75
log(Absorbance)
2.55
2.60
2.65
log(Wavelength)
CK15 40%PEG/TFA
CK45 40%PEG/TFA
CK45 100%PEG/Acetate
CK30 100%PEG/TFA

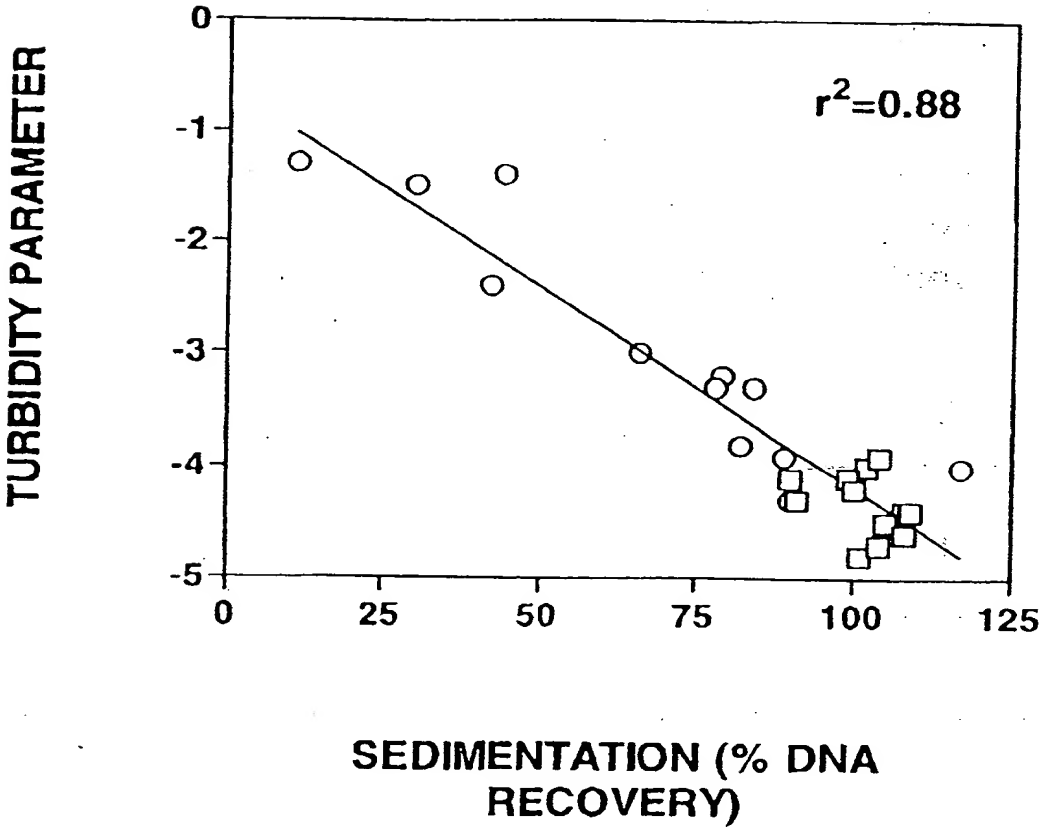
Fig. 8



○ Type A Formulations

□ Type B Formulations

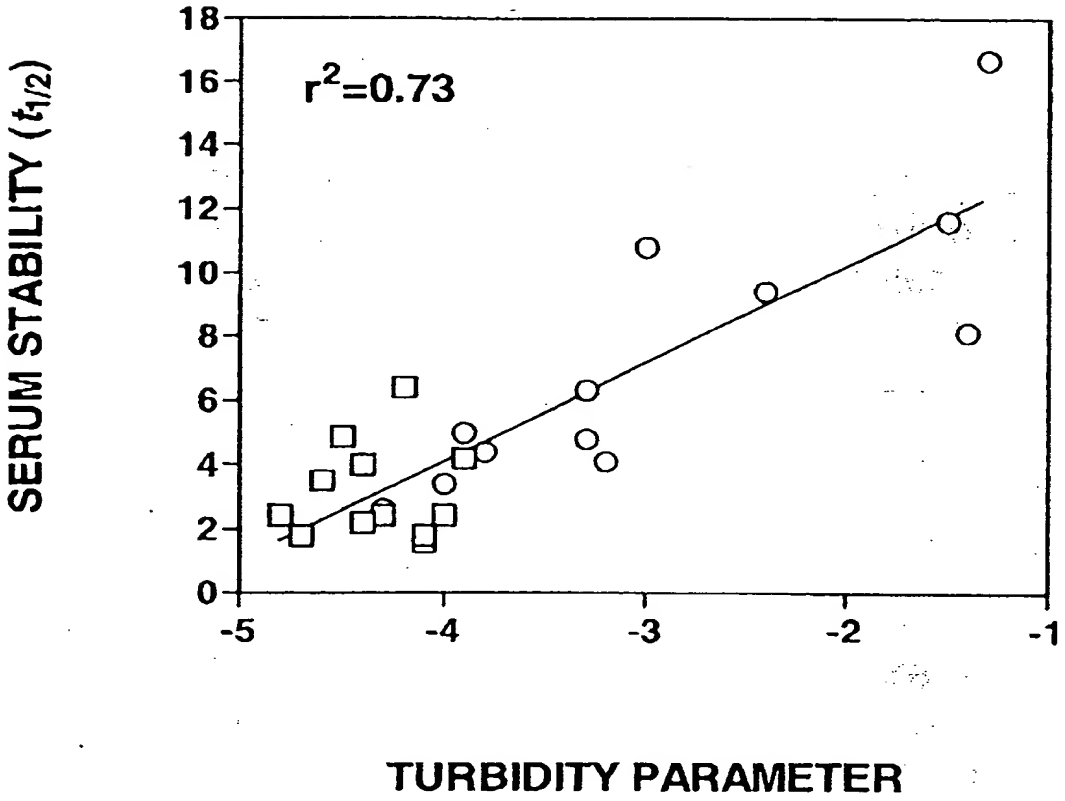
Fig. 9A



○ Type A Formulations

□ Type B Formulations

Fig. 9B



○ Type A Formulations

□ Type B Formulations

Fig. 9C

PROPERTIES OF VARIOUS PLAS*min*™ FORMULATIONS

Formulation #	Counterion	Polylysine	PEG Content (%)	$t_{1/2}$ In Serum (h)	Turbidity Parameter	Sedimentation (%)
1	TFA	CK_{15}	40	11.6	-1.5	30
2			60	10.8	-3.0	66
3			80	9.4	-2.4	42
4			100	16.7	-1.3	11
5	TFA	CK_{30}	40	8.1	-1.4	44
6			60	4.1	-3.2	79
7			80	3.4	-4.0	117
8			100	2.6	-4.3	90
9	TFA	CK_{45}	40	6.3	-3.3	78
10			60	4.4	-3.8	82
11			80	4.8	-3.3	84
12			100	5.0	-3.9	89
13	Acetate	CK_{15}	40	2.4	-4.8	101
14			60	1.8	-4.7	104
15			80	1.6	-4.1	90
16			100	2.4	-4.0	102
17	Acetate	CK_{30}	40	1.8	-4.1	99
18			60	2.4	-4.3	91
19			80	2.2	-4.4	108
20			100	4.0	-4.4	109
21	Acetate	CK_{45}	40	6.4	-4.2	100
22			60	4.2	-3.9	104
23			80	4.9	-4.5	105
24			100	3.5	-4.6	108

Fig. 9D

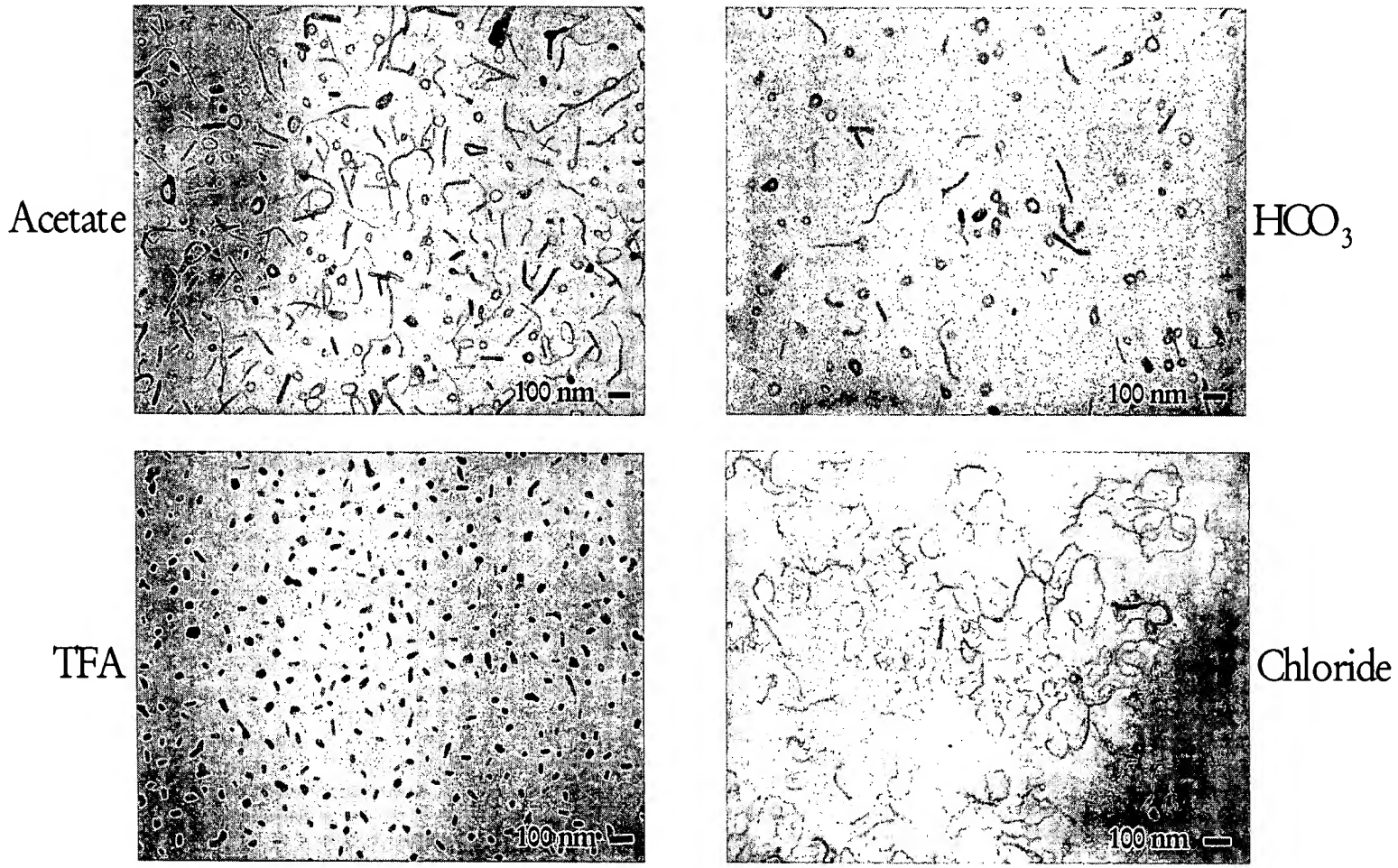


Fig. 10

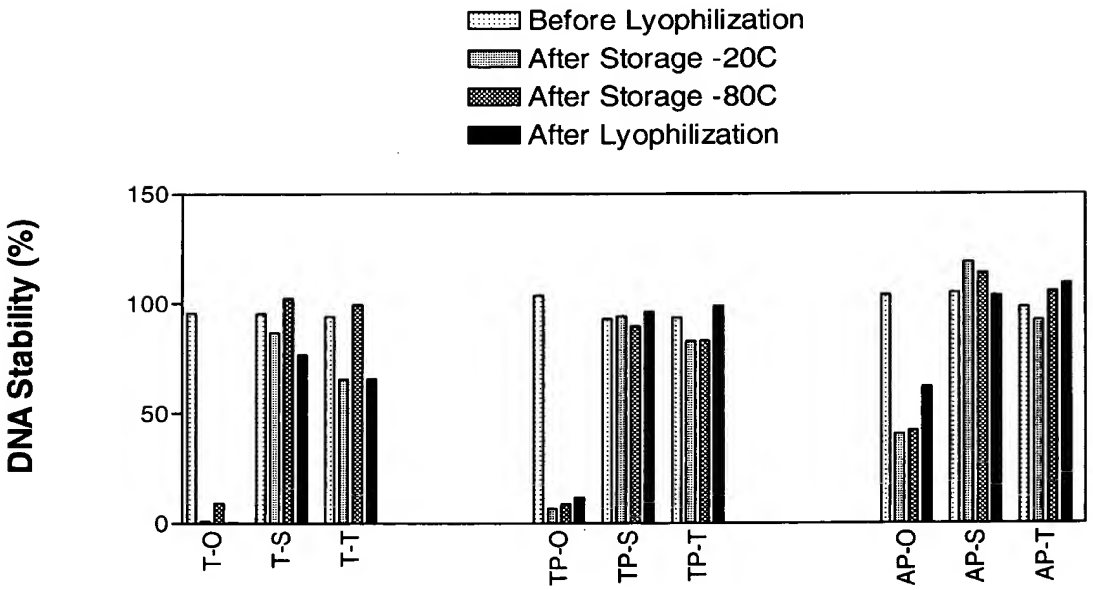
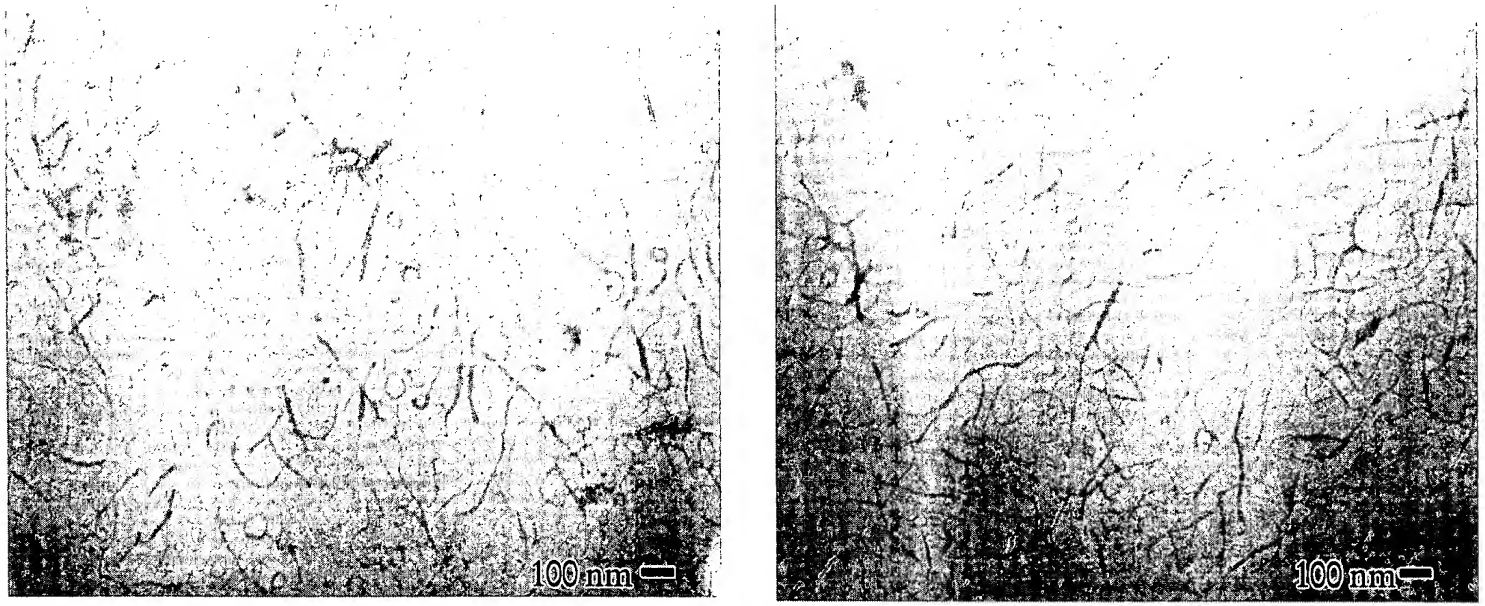

Before Lyophilization
After Storage -20C
After Storage -80C
After Lyophilization
DNA Stability (%)
150
100
50
0
T-O
T-S
T-T
TP-O
TP-S
TP-T
AP-O
AP-S
AP-T

Fig. 11

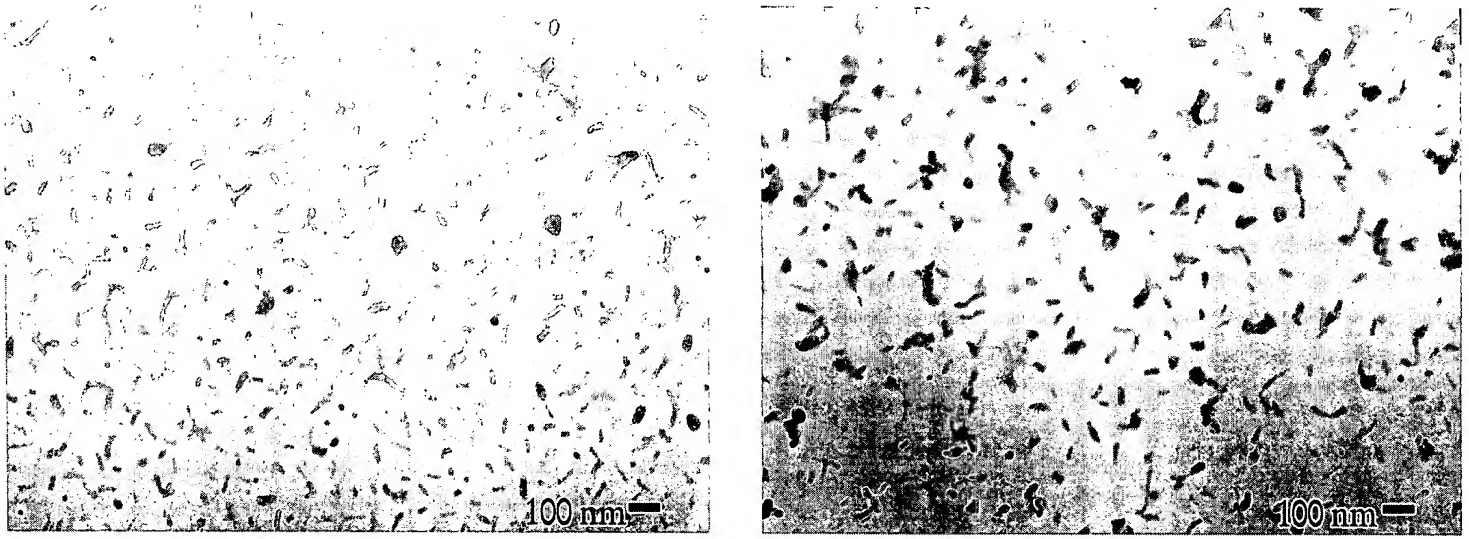
Sample	Before Lyophilization	After Lyophilization
CK30TFA		
Original		
0.5M Sucrose	-4.31	ppt
0.5 M Trehalose	-3.81	-4.10
CK30P10k - TFA	-4.70	-4.01
Original		
0.5M Sucrose	-4.51	NE-4.61
0.5 M Trehalose	-4.15	
CK30P10k - Acetate	-4.65	-4.66
Original		-3.86
0.5M Sucrose	-4.76	
0.5 M Trehalose	-4.56	-3.32
	-4.57	-4.39

Fig. 12



BEFORE AFTER

Fig. 13



BEFORE **AFTER**

Fig. 14

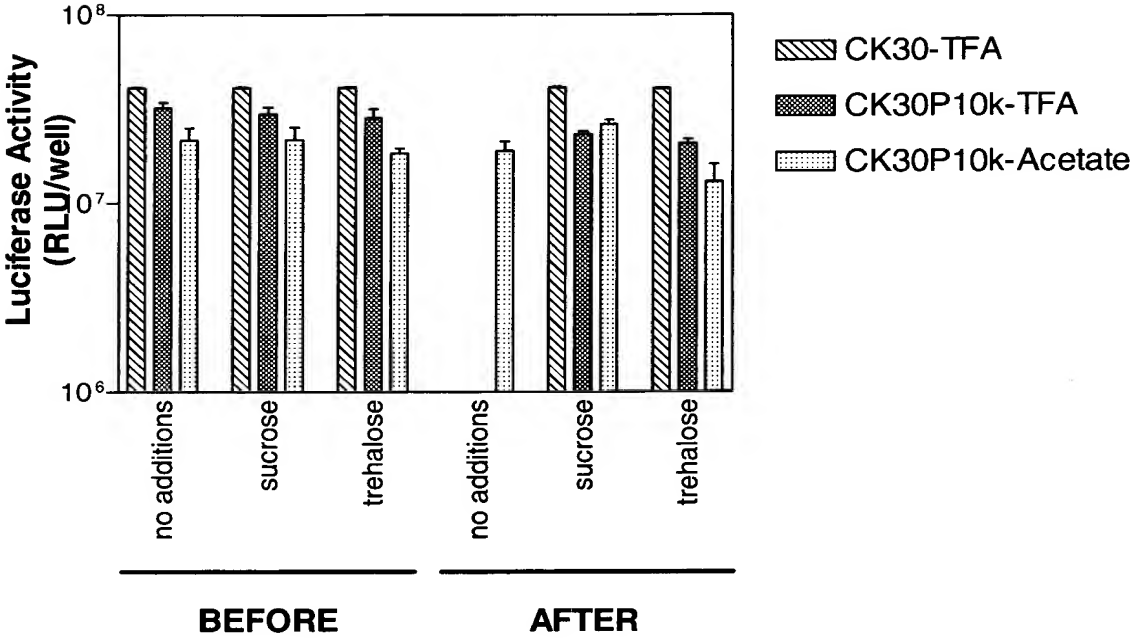

Luciferase Activity (RLU/well)
10^8
10^7
10^6
CK30-TFA
CK30P10k-TFA
CK30P10k-Acetate
no additions
sucrose
trehalose
no additions
sucrose
trehalose
BEFORE
AFTER

Fig. 15

Polylysine	Counterion	DNA Recovery	Turbidity Parameter
CK30P10k	Acetate	100	-4.2
	Bicarbonate	98	-4.0
	Chloride	101	-5.2*
	TFA	97	-4.6
CK45P10k	Chloride	105	-4.0

* This value is lower than expected due to very low light scattering by this DNA formulation indicating that plasmid is not compacted, in agreement with electron microscopy and gel electrophoresis data.

Fig. 16

Magnification 40,000. The bar shows 100 nm

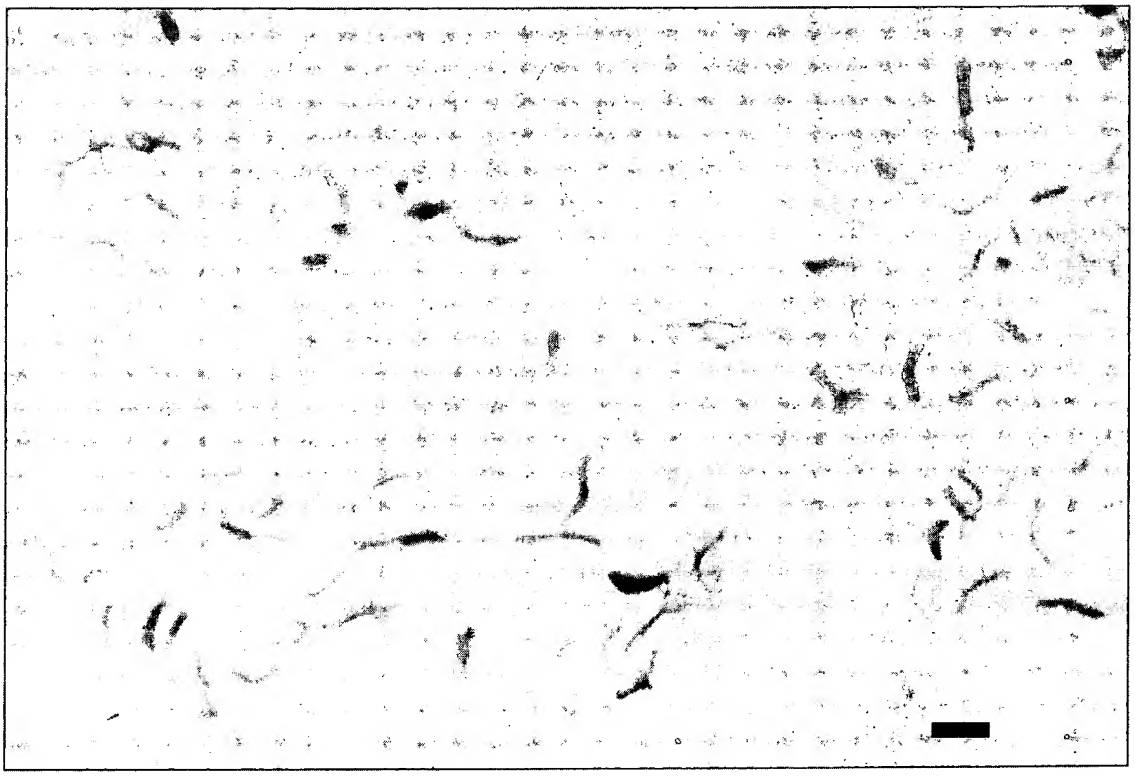


Fig. 17

Lane 1: DNA size markers.
Lane 2: naked DNA before compaction.
Lanes 3, 6, 9, and 12: compacted DNA.
Lanes 4, 7, 10, and 13: compacted DNA that was incubated in 75% mouse serum at 37 °C for 2 hr and trypsinized before loading.
Lanes 5, 8, 11, and 14: compacted DNA that was only trypsinized before loading.

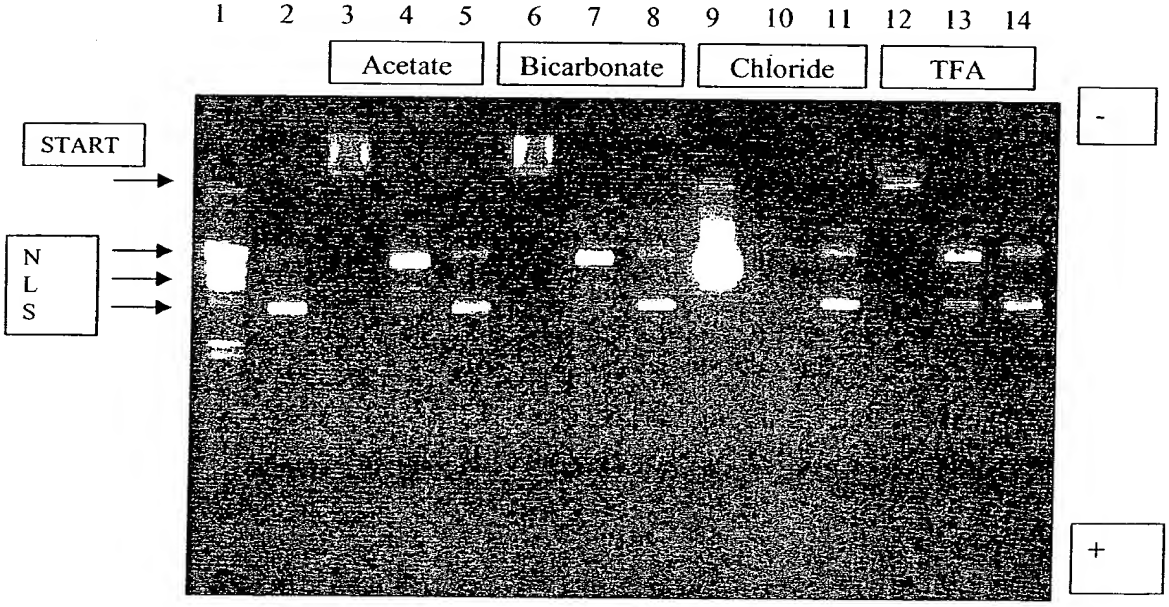
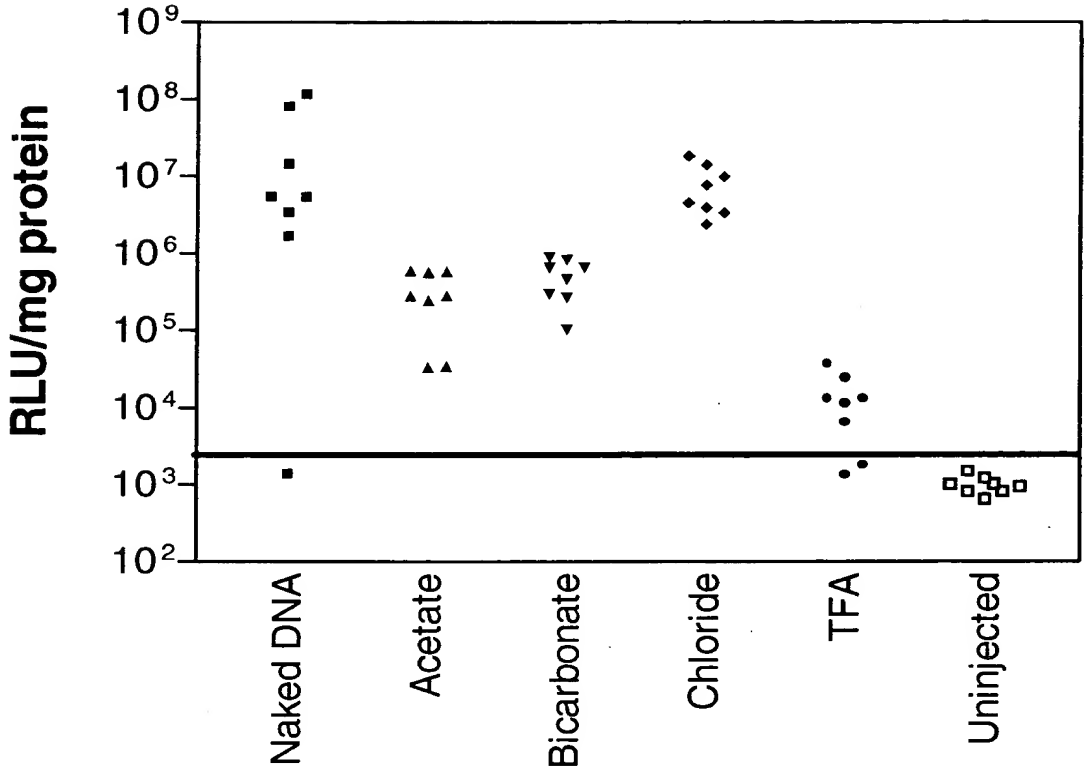
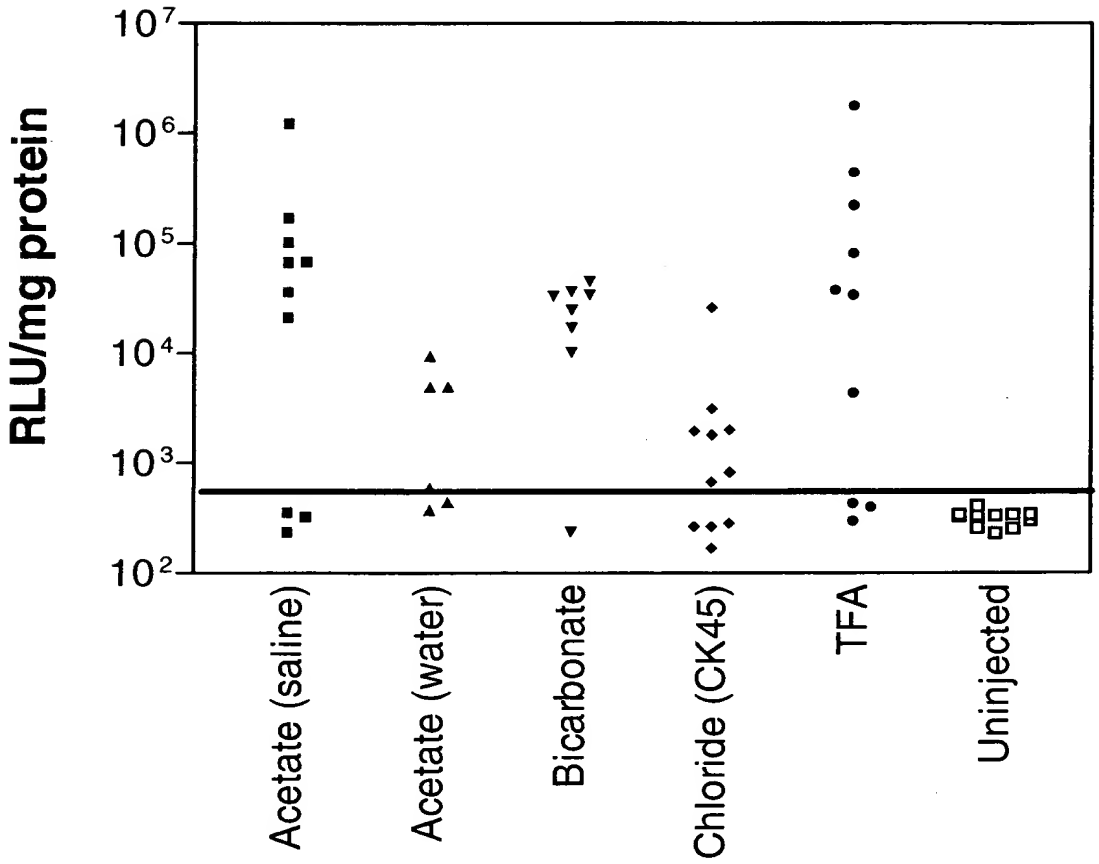


Fig. 18



Each point represents one animal. The solid line indicates background signal of luciferase assay. Dose 100 μg DNA.

Fig. 19



Each point represents one animal. The solid line indicates background signal of luciferase assay. Dose 100 µg DNA.

Fig. 20